

## **STRUCTURAL AND FUNCTIONAL GENOMICS APPLIED TO AN ANTHOCYANIN-FREE EGGPLANT GENOTYPE FOR THE ANALYSIS OF COLOUR REGULATION IN PEEL**

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*anthocyanin, RNA-seq, genome re-sequencing, eggplant, epigenetic regulation*

In the eggplant (*Solanum melongena* L.) purple fruits varieties, anthocyanins are synthesized in the fruit peel. In a previous study (Gisbert et al., 2016, *Hortscience* 51:793–798), a green-fruited eggplant plant originated by spontaneous mutations from the violet-fruited ‘Black Beauty’ (BB) was identified, and from this a new variety named ‘Green Beauty’ (GB) selected. Loss of anthocyanin structural gene function in the green-fruited eggplant mutant was associated with reduced levels of MybC and/or Myc regulatory gene transcripts.

With the goal to shed light on the molecular mechanisms responsible for the berry green phenotype, the sequencing of RNA extracted from the peel of both the BB and GB varieties as well as their genome re-sequencing were performed.

RNA sequencing data highlighted a set of 7,458 genes differentially expressed (DEGs,  $\pm 1$  fold) in the peel of GB in respect to BB, of which 68.76% were up-regulated, while 31.24% down-regulated.

Resequencing data identified in GB a total of 842 moderate impact SNPs/indels, of which 150 in DEGs, while a total of 97 deleterious SNPs/indels were detected, of which 11 in DEGs. The structural variation (SV) analysis performed with Pindel (<https://github.com/genome/pindel>) revealed 20,528 SV of which 14,989 in DEGs. The Delly analysis (<https://github.com/dellytools/delly>) highlighted 268 SV of which 103 in DEGs.

In order to identify putative mutations responsible for the GB phenotype, attention was focused on deleterious polymorphisms (SNPs/SV) in DEGs. By considering only homozygous mutations, 5 candidate SNPs and 1 SV were found, and due to evidences previously reported in literature (Fan et al. 2018, *Plant J.*, 96:1121-1136), the *SUVH5* (Histone-lysine N-methyltransferase, specific for H3 lysine-9) was selected as candidate gene.

The SUVH5 protein contributes to the maintenance of histone H3 methylation at the level of lysine 9 (H3K9me1/me2) and to the CMT3-mediated methylation of DNA of non-CG sites. In GB plants, *SUVH5* showed a deleterious deletion of 591 bp located downstream the gene, causing the loss of the stop codon and the ablation of one of the four key cysteines at the C-terminal level. As highlighted by 3D protein modelling

analysis, its substitution with a serine residue can abolish the methyltransferase activity of the protein, resulting in the loss of H3K9me2 methylations and DNA methylations at other specific target regulatory genes (e.g.: MYB-like). The latter were further investigated through quantitative gene expression analysis to clarify their regulation in the anthocyanin pathway in peel.

The present results suggest that *SUVH5* is a candidate gene likely contributing to the peel colour regulation in the GB variety through an epigenetic mechanism.